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U.S. EPA
Document Control Officer (7407M)
1200 Pennsylvania Ave., NW
Washington, DC 20460
Attn: HPV Challenge Program.
Docket Control Number OPPTS-42213
Administrative Record Number AR-201

Re: High Production Volume (HPV) Challenge Program – Data Analysis and Test Plan for Resorcinol

AMEC is submitting comments to EPA on the above referenced document. We have identified several errors in reporting the toxicity information in Sections 1, 3, 5, 6 and 7 to which we would like to draw your attention.

Section 1 – Executive Summary

The section on human health provides information about *in vitro* and *in vivo* mammalian genotoxicity studies, but it does not report that resorcinol is not mutagenic to bacteria or Drosophila.

The section on ecotoxicity cites a lowest no effect concentration (NOEC) of 172 µg a.i./l (NOEC, full life cycle toxicity test for Daphnia magna) for resorcinol (Lima, 2004). It is not clear what the toxicological significance of the lowest NOEC is in evaluating the potential toxicity of resorcinol. This value was the maximum study concentration, and it did not cause any effects. Higher concentrations were not tested, so the LOEC concentration could not be determined.

Higher NOECs than 172 ug/L have been reported from studies that were performed at higher concentration levels. For instance, Hoechst (1981) (Reference 23) reported a 48 and 96 hour NOEC of 25 mg/L for Golden Ide (*Leuciscus idus f. melanotus*). Curtis et al. (1978) (IUCLID Reference 24) reported a 48 hour NOEC of 72.6 mg/L for Pimephales promelas. Stom and Zubareva (1994) reported a NOEC of 11 mg/L for *Daphnia* (11 mg/L).

AMEC recommends that the relevant test results to summarize the aquatic toxicity of a compound are the highest no effect level and the lowest effect level, not the lowest no effect level. For resorcinol, the highest no effect level in fish is 72.6 mg/L and the lowest low effect level in fish is 26.8 mg/L.

Section 3 – Physicochemical Properties

The results for partition coefficient are incorrectly labeled Kow. They should be log Kow.

Section 5 – Ecotoxicity Data

Table 6 fails to list the 48 hour NOEC for Pimephales promelas from IUCLID reference #24 (72.6 mg/L).

Table 7 incorrectly lists 0.25 mg/l as a LC50 for Daphnia magna. It is an EC50 as noted in the IUCLID summary for the study.

The report states:

> "However all the studies demonstrate that Resorcinol is toxic to fish in varying degrees (see Table 6 for a summary)."

The statement is misleading, because all chemicals are toxic to fish at sufficiently high concentrations. In fact, resorcinol is not very toxic to fish compared to many other chemicals present in the environment. Table 6 indicates that the concentrations of resorcinol as high as 25 mg/L are associated with no effects in fish and the lowest reported effect concentration is an LC50 of 26.8 mg/L for fathead minnows. In addition, data from IUCLID reference 24 reports a NOEC of 72.6 mg/L for fat head minnows.

AMEC (2003) has reviewed the toxicological data for resorcinol and found that sufficient data were available to satisfy seven of the eight data requirements to calculate ambient water quality criteria according to USEPA protocols (Stephan et al., 1985). The AMEC Bioassay Laboratory gathered additional toxicity information to fill the last data requirement and calculated acute (25 mg/L) and chronic (7 mg/L) values for the protection of aquatic life (AMEC, 2003).

Review of all current USEPA acute and chronic ambient water quality criteria for the protection of aquatic life for all chemicals shows that acute criteria range from 0.014 ug/L to 570 ug/L, and chronic criteria range from 0.0002 ug/L to 150 ug/L. The maximum USEPA acute and chronic ambient water quality criteria are 44 and 46 times lower than respective resorcinol values. Thus, while very high concentrations of resorcinol are toxic to aquatic organisms, resorcinol is much less toxic than other compounds currently regulated by USEPA.

The IUCLID data summary appended to the report cites an unpublished study by Lima (2004), which is summarized on page 12 of the HPV report as follows:

> This study demonstrated that concentrations up to 172 µg a.i./l of Resorcinol had no adverse effects on survival, growth or reproduction of Daphnia magna. LOEC was determined to be >172µg a.i./l. This study clearly demonstrates that Resorcinol is very toxic to aquatic organisms, and so must be classified R50. This study can also be used to provide data for the acute toxicity endpoint as observations were made for mortality and effects on a daily basis for 21 days. After 48 hours EC>172 µg/l and NOEC = 172 µg/l.

However, review of the information presented in the data summary indicates that the maximum study concentration did not cause any effects, so the LOEC concentration could not be determined. Further, the true NOEC for this compound is far higher than the concentration tested, which is extremely low. It is not correct to state that this "study clearly demonstrates that Resorcinol is very toxic to aquatic organisms, and so must be classified R50." The criteria for the R50 designation requires an acute LC50 that is less than 1 mg/L, and the chronic criteria requires that the LC(EC)50 is less than 1 mg/L and that the substance is not rapidly degradable and/or the log Kow is greater than 4 (Lee-Steere, 2004). Since this study did not observe any effects at the concentrations tested and since the log Kow has been measured at 0.97 at 20C (Beezer et al., 1980) and estimated to be 1.03 (USEPA, 2000), it fails all requirements to be designated as "R50."

Section 6 - Mammalian Toxicity

In Table 8, "Repeated Dose", the exposure duration for the short term NTP gavage study for rats and mice was reported as 14 days rather than 17 days. NTP refers to these studies as "17-day studies" despite the fact that the animals were dosed 5 days a week over the period resulting in 12 total dosages. "No toxic effects" was erroneously reported for this 17-day rat gavage study, and 450 mg/kg-day was listed as a NOEL. In reality, NTP (1992) reported several different effects at different dose levels, as noted below. At 450 mg/kg-day, increased mortality and statistically significant effects on body weight were reported. Clearly, 450 mg/kg-day is not a NOEL with a reported endpoint of increased mortality. The IUCLID database also misreported this NOEL. In addition, at 225 mg/kg-day, statistically significant decreases in thymus weight in female rats was reported. Hence, AMEC recommends that 225 mg/kg/day (thymus weight changes) be defined as the NOEL for this study.

With regard to the 17-day mouse study (NTP, 1992), the NOEL of 100 mg/kg/day reported for the mouse study should be 150 mg/kg/day. No 100 mg/kg/day dose was used in the 17-day mouse study. The IUCLID database also misreported this NOEL.

It is not clear what is meant by "Not classified" as reported under "Remarks" for the NTP 13-week study. NTP (1992) did report effects. In rats, the effects noted include mortality, changes in body weight, and changes in liver and adrenal weights. In mice, the effects noted include mortality, changes in body weight, and changes in adrenal weights. The NOEL reported for rats was 260 mg/kg-day. This is the NOEL for body weight changes and mortality. However, the NOEL for changes in liver weight is 32 mg/kg-day. For adrenal weight changes, there was no NOEL. The LOEL was 32 mg/kg-day. The NOEL reported for mice was 225 mg/kg-day. This is the NOEL for body weight changes and mortality. However, there is no NOEL for changes in adrenal weight. The LOEL is 28 mg/kg-day for adrenal weight changes. AMEC notes that the effects on adrenal weight may not be adverse effects, because there were increases in adrenal weight changes in rats and decreases in adrenal weight changes in mice.

Dosing in the NTP gavage studies did not occur every day throughout the study. The reported NOELs in Table 8 were nominal doses that are not adjusted for this less-than-daily dosing.

Under "Genetic Toxicity – *in vivo*", reference 16 (Flickinger et al., 1976) is cited as the reference for the Micronucleus assay. This is incorrectly cited. Genetic toxicity testing is not discussed in Flickinger et al. (1976). This is most likely an error in transcribing the record from the IUCLID database. The IUCLID database does contain a citation from Flickinger et al. (1976) (IUCLID reference number 37), but the database does not use this reference to cite genetic toxicity. IUCLID reference 137 supports a negative result from a Micronucleus assay and is most likely what is being reported in Table 8. IUCLID reference number 137 is Wild et al. (1981), which is also cited in Table 8 as the reference for "Sperm abnormality".

In Table 8, "Developmental Toxicity/Teratogenicity", reference is made to a "Rat, Day 1-19 gestation" study. The 2 ml/kg given as the dose is the volume of a hair dye mixture applied dermally to the shaved backs of rats in the study. To be consistent, this application volume should be converted to a dose. Application occurred on days 1, 4, 7, 10, 13, 16 and 19 of gestation. It should be noted that this was a dermal study, whereas the other studies were oral studies (as noted under "Remarks"). In addition, it should be noted that a hair dye mixture was applied; resorcinol alone was not evaluated.

The entry in Table 8, "Rabbit, Day 6-15 gestation, 40-250 mg/kg, Reference 37 is incorrect. The correct entry for this study precedes this incorrect entry, and is reported as "Rabbit, Day 6-18 of gestation, 25-100 mg/kg, Reference 18". Reference 37, Spengler et al. reports the same rat and rabbit studies that are cited in Table 8 as "Reference 18," the Hazelton Laboratory reports. Similarly, the IUCLID database erroneously reports a "Rabbit, Day 6-15 gestation, 40-250 mg/kg study and references it "as cited in NTP (1992)". In fact, there no such study in which rabbits were given 40-250 mg/kg during days 6-15 of gestation. This entry in Table 8 should be removed.

The units for the dose for "Hen chick eggs single dose" are incorrect. The dose should be reported as 99 - 804 µg/chick egg rather than mg/chick egg.

In Section 6.2 (Repeated Dose Toxicity), it is stated that "No chemical-related gross or microscopic lesions were observed in either study," when discussing the 17-day and 91-day NTP studies in rats and mice. This statement should be revised to note that mortality was seen in various high dose groups, and other dose groups were reported to have changes in body weight, thymus weight, liver weight and adrenal weight. Specific dose levels at which these effects were reported have already been discussed above.

In Section 6.5 (Developmental Toxicity), the sentence should be revised to say "...following oral or dermal administration." Otherwise, reference 8 should be removed from this section and Table 8.

Section 7 – "Beyond SIDS" Endpoints

Reference 45 (Van Leewen et al., 1990) is apparently a mis-citation. Van Leewen et al. (1990) is an ecotoxicity paper. It is not a carcinogenicity study in mice. AMEC suspects that the authors intended to cite Van Duuren and Goldschmidt (1976), which is IUCLID reference 130 versus IUCLID reference 131, which is Van Leewen et al. (1990).

The third paragraph contains several errors regarding the conclusions of the cited studies. Of all the studies cited as the basis for the statement that resorcinol has a "minor promotional effect," only one supports the conclusion. Yamaguchi et al. (1989) (Reference 47) found that resorcinol given in the diet for 49 weeks (728 mg/kg-day) promoted the effect of the carcinogen methyl-n-nitrosamine given by intraperitoneal injection on tongue papillomas and esophageal squamous cell carcinomas in rats.

However, all of the other citations are negative or equivocal. First Boutwell and Bosch (1959) (Reference 4) concluded that resorcinol did *not* have promoting activity in mouse skin. While it is true as noted in the IUCLID entry that none of the control animals had papillomas or carcinomas, none of the treated animals had carcinomas, and 17% of the treated animals had papillomas, the control rate in other of the experiments reported in the paper range from 7% to 13%. For this reason and because the number of papillomas per animal was low, the authors concluded that resorcinol had "questionable activity." Secondly, Van Duuren and Goldschmidt (1976) (intended Reference 45), did not seen a promotional effect with resorcinol. Their study was negative for promotion.

Further, Hirose et al. (1989) (Reference 20) concluded that resorcinol did *not* increase the incidence of forestomach carcinomas in the initiated group. Lastly, Maruyama et al. (1991) (Reference 30) reported that resorcinol, as well as other phenolics tested, had an *inhibitory* effect on pancreatic carcinogenesis in hamsters, not a promotional effect.

Other studies that are not cited in the IUCLID entry also support the conclusion that resorcinol in not a tumor promoter. Resorcinol alone did not induce bladder lesions nor did it enhance any tumor after the administration of the initiator (Miyata et al., 1985). No promotional effect of resorcinol was seen in rats pretreated N-nitrosodiethylamine, an initiator of liver carcinogenesis (Stenius et al., 1989). Resorcinol alone did not induce bladder tumors nor did resorcinol increase the incidence of bladder lesions in rats initiated with the bladder carcinogen, N-butyl-N-(4-hydroxybutyl)nitrosamine (Kurata et al., 1990).

AMEC thus recommends that this text be revised to state that resorcinol does *not* appear to have a promotional effect.

Best regards,

Brian, Magee Ph.D.
Vice President, Principal Toxicologist
AMEC Earth & Environmental, Inc.

References

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